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Effect of food concentration on the growth and production rate of *Artemia franciscana* feeding on algae (*T. iso*)

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Abstract

Individual dry weight and carbon content, ingestion rate, growth rate and production rate were determined for different developmental stages of *Artemia franciscana* feeding on six different concentrations of *Isochrysis galbana* (Tahiti). The cultures were grown at nearly constant food concentrations, ranging from 0.2 to 20 mg C l^{-1} for 12 days at a temperature of 26–28°C and 34 ppt salinity. The individual body dry weight increased exponentially with age to a point of inflection before the growth rate gradually decreased when the animals reached the pre-adult stage. The growth of *A. franciscana* was strongly influenced by the food concentration. Minimum food concentration required for maximum growth was 10 mg C l^{-1}. At this food concentration the individual dry weigh increased from 2.3 μg ind^{-1} (newly hatched nauplii) to 195±7.03 μg ind^{-1} (mean±SE) when the animals had reached pre-adult stage (11 days growth). In the same period *A. franciscana* grown at lower food concentrations, 7, 5 and 3 mg C l^{-1}, reached 134±3.41, 88±3.53 and 29±3.09 μg (mean±SE), respectively. At the lowest food concentration tested (0.2 mg C l^{-1}) the individual dry weight was reduced by 14–18% after 5 days of cultivation, indicating that the animals were starving. The carbon and nitrogen content (% of dry weight) of newly hatched nauplii was 45.5 and 10.1%, respectively. During the growth period the carbon content varied between 40.1 and 45.5%, and the nitrogen content between 9.1 and 10.5%. The specific growth rate (μ, day^{-1}) and the ingestion rate (ng C ind^{-1} day^{-1}) were strongly affected by the size of the animals and the food concentration. Both rates increased with increasing food concentration and reached a maximum level at an algal concentration of 10 mg C l^{-1}. The specific growth rate and the ingestion rate were not influenced by an increase of the food concentration above 10 mg C l^{-1}. At these food concentrations the specific growth rate showed a maximum value when the animals were in the range 6–51 μg ind^{-1} (μ_{max} = 0.9–1.15 day^{-1}). The weight specific ingestion rate (I_W, h^{-1}) also increased with increasing food concentration. *A. franciscana*

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in the range of 7–20 μg ind^{-1}, grown at food concentrations ≥10 mg C l^{-1}, ingested on carbon basis approximately 20–28% of the individual carbon content per hour (I_W = 0.20–0.28 h^{-1}). At the lower food concentrations (3 and 5 mg C l^{-1}) the ingestion rate was reduced and the weight specific ingestion rate varied between 0.05 and 0.1 h^{-1}. The production rate (P_r, μg C ind^{-1} day^{-1}) showed a distinct maximum for animals grown at the two highest food concentrations. When individual dry weight exceeded 6 μg ind^{-1} (post-metanauplius II) animal size affected both the production rate and the relative production rate (%, day^{-1}). Thereafter the food concentration had a more pronounced effect on the production rate of the animals. The conversion of ingested food into biomass (yield, day^{-1}) was dependent on food concentration and animal size. Animals fed at the lowest food concentrations (3, 5 and 7 mg C l^{-1}) attained a yield of 0.50–0.65 at post-metanauplius stage III, and remained at this level until post-metanauplius stage VII (dry weight; 10–50 μg ind^{-1}). Animals at the same size but fed at the highest food concentrations (10 and 20 mg C l^{-1}), showed a yield close to 0.15–0.20 at post-metanauplius stage III increasing to 0.50 at post-metanauplius stage VI–VII. In the older stages the yield decreased from a mean of 0.46 to 0.05–0.10 when the animals attained pre-adult stage. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Artemia; Food concentration; Growth rate; Production rate

1. Introduction

In the pelagic community the ingestion rate of the herbivorous zooplankton is one of the factors that regulate the mass of phytoplankton and so influences the production rate of the whole ecosystem (Tait, 1983). Since Rigler (1961) described an incipient limiting food concentration for *Daphnia* many field and laboratory studies have demonstrated that the algal concentration has a striking effect on most of the planktonic filter feeders. The ingestion rate, the growth rate and the carbon balance of different zooplankton species are highly affected by the availability of food (Sushchenya, 1962; Frost, 1974; Geller, 1975; Lampert, 1977a and b; Mullin and Brooks, 1970; Checkly, 1980; Porter et al., 1982; Krylov, 1988; Urabe, 1988; 1991; Evjemo et al., 1999). Several studies on *Daphnia* have shown that the growth rate increases with increasing food concentration (Lampert, 1977a; Lei and Armitage, 1980; Porter et al., 1982; Lynch et al., 1986), findings which have been further supported by several authors working with copepods (Mullin, 1963; Mullin and Brooks, 1970; Paffenhöfer and Harris, 1976; Miller et al., 1977; Vidal, 1980).

Since planktonic copepods are the most important filter feeders in the oceans and the cladocerans play a prominent role as primary consumers in many freshwater ecosystems, less attention has been paid to other zooplankters, such as the anostracan and the family of the *Artemiidae,* where less information exists on the growth rate and the ingestion rate of specific populations. *Artemiidae* are found in saline lakes and brine ponds all over the world. The largest population is found in Great Salt Lake, UT, USA, where *Artemia franciscana* is the only dominant planktonic invertebrate species in the lake. The pelagic energy flow system is quite simple, with interactions between *A. franciscana*, several algal species, protozoa and bacteria (Stephens and Gillespie, 1976). During the last

decades the population has been exploited for industrial purposes. The quiescent cysts, formed by the animals at certain times of the year, are harvested by man and distributed world wide for aquaculture purposes. It is realised that future over-exploitation of the stock may be a problem for the future supply of cysts.

The *Artemiidae* are considered to be a continuous, non-selective, obligate phagotrophic filter feeder (Provasoli and Shiraishi, 1959; Reeve, 1963b; D'Agostino, 1980) which starts to ingest food at Instar II stage (metanauplius I) using the larval antennae (Barlow and Sleigh, 1980). During the post-embryonic development the feeding function is gradually taken over by the multifunctional thoracopods (Schrehardt, 1987), which are fully developed at the pre-adult stage (Blanchard, 1987). Moreover, it has been shown that the ingestion rate changes as a function of food concentration according to a Type 3 functional response (Evjemo et al., 1999) or to a rectilinear Type 2 of functional response curve (Reeve, 1963a; Yanase and Shiraishi, 1972; Coutteau, 1992). Similar feeding behaviour has been well documented for both *Daphnia* (Rigler, 1961; Geller, 1975; Horton et al., 1979; Porter et al., 1982) and copepods (Munro, 1974; Mullin et al., 1975; Frost, 1975; Vidal, 1980).

In terms of energy flow, ingestion represents the greatest of all interactions between animal and its environment (Spomer, 1973), and the concept of material flow through the food webs has been applied both in limnetic and in the marine ecosystems. Much work has been carried out in order to quantify energy transfer, particularly interactions at the lower trophical levels of the pelagic ecosystem. Since production estimates requires a quantitative evaluation of the effect the phytoplankton concentration have on the consuming filtering zooplankton, information on the rates of feeding, growth, metabolism and production are essential for evaluating the impact of grazing on phytoplankton populations. The zooplankton body size and the type of grazers have shown to strongly influence the distribution and the community structure of the algae (Bergquist et al., 1985), and regression models developed by Peters (1984) have shown that zooplankton size is the most important variable for prediction of the grazing rate. In situ studies have demonstrated that grazing rates are largely dependent on the herbivore biomass (Chow-Fraser and Knoechel, 1985; Knoechel, 1977; Knoechel and Holteby, 1986; Lampert, 1987) and the zooplankton grazing pressure can be sufficiently high to reduce algal concentration and promote change in the algal community structure, and cause seasonal succession within the whole plankton community (Porter, 1973, 1977; Crumpton and Wetzel, 1982; Sommer et al., 1986; Kerfoot, 1987). Growth experiments on marine planktonic copepods have suggested that growth rate is proportional to food concentration (Vidal, 1980), but also influenced by the quality of food (Mullin and Brooks, 1970; Paffenhöfer and Harris, 1976) and the temperature (Landry, 1976). An especially important environmental factor for filter feeders is therefore food concentration (Rigler, 1961; Frost, 1972; Geller, 1975; Vidal, 1980; Porter et al., 1982; Urabe, 1991), and according to Lampert (1977a) the most important environmental factor influencing the production of *Daphnia*. Since the availability of food has a strong influence on the zooplankton production rate, the algal concentration has an indirect effect on the production on higher trophical levels of the ecosystem.

The knowledge on the response of planktonic filter feeders on variable food concentrations is of great importance for the understanding of energy transfer. For

different populations of the *Artemiidae* little information exists on the rates of growth and production at variable food concentrations. Estimates of the ingestion rate, growth and production rate are valuable for making predictions of biomass production in a population.

The present study was undertaken to establish quantitative knowledge on changes in the growth and production rates when *A. franciscana* was grown from nauplii to pre-adult stage under different concentrations of algae. Based on growth experiments the present paper describes changes in the growth, production and ingestion rates and determines the food concentrations corresponding to where the growth rate is maximal and were the growth rate is limited by the food intake.

2. Material and methods

The individual dry weight, the carbon and nitrogen content, the body length and the survival rate of *A. franciscana* were determined in six separate cultures feeding on different, but nearly constant, food concentrations of *I. galbana* (0.2, 3, 5, 7, 10 and 20 mg C l^{-1}) for 12 days (1 mg C l^{-1} corresponds to $130 \cdot 10^6$ cells l^{-1}; Reitan et al., 1993). During this period the growth rate and the ingestion rate (ng C ind^{-1} h^{-1}) were determined every 24 h. The ingestion rate was estimated by giving the animals a pulse meal of ^{14}C-labelled *I. galbana* at the same concentration as the animals were grown at in the culture tanks.

2.1. Animal rearing

The experiments were performed with cysts from *Artemia franciscana*, originating from Great Salt Lake, UT, USA (INVE Aquaculture, Belgium). The cysts were hydrated, decapsulated, hatched (Sorgeloos et al., 1986) and nauplii were transferred to six different 40 l conical polyethylene tanks and incubated (day 0) at densities ranging from 16 to 18 ind ml^{-1}. From day 2 until day 3 the animal densities were diluted to 4.2–6.3 ind ml^{-1}, due to a reduction of the two highest food concentrations by approximately 7 and 8%. The next 24 h (day 3–4) the food concentrations in the same cultures were reduced by 4 and 6%, and in order to keep the food concentrations nearly constant, the animal densities were further reduced to 1.1–2.4 ind ml^{-1}. From this day (day 4) until the end of the experiment the densities were gradually reduced to 0.48–0.81 ind ml^{-1}. This reduction was related to sampling of *A. franciscana* for the dry weight analysis (carbon–nitrogen analysis), and animals for the feeding experiments with labelled algae. In this part of the experiment (day 4–day 12) the food concentration in the cultures did not change more than 2%, an exception was when the water in the cultures was changed (see below).

The cultivation tanks were filled with membrane-filtered sea water (2 μm) from a reservoir, at a salinity of 34 ppt and a temperature of 26–28°C. The water in the reservoir (600 l) was heated with a thermostat controlled heating element (2000 W). In the culture tanks the water was kept at a temperature of 26–28°C by the use of thermostat controlled aquarium heater (300 W). The cultures were aerated by the use of

diffusers to obtain $[O_2]>3.5$ mg l^{-1}. Daily measurements of $[O_2]$ showed that it was in the range of 6.7–7.4 mg l^{-1} during the experiment. The light intensity was measured 10 cm above the surface of the culture tanks and found to be constant throughout the experimental period (60 μEin m^{-2} s^{-1}).

A. franciscana was fed every 12th hour. During daytime, the food concentrations were measured from three samples three times a day using spectrophotometer (Shimadzu model UV-150-02), and if necessary, adjusted by adding more algae. These samples were sieved (20 μm) to remove faecal pellets from the animals. Culture water was exchanged every other day using a flow through-system which kept the water level of the tanks constant. During the water exchange, the aeration was stopped for 6 min to allow dead animals, faecal pellets and exoskeletons to sediment and concentrate on the bottom of the tanks. The organic matter was drained before exchanging the water. During the next 30 min, the water in the cultures was diluted several times with filtered, temperated sea water (28°C). During this procedure, the algal concentration of the cultures became less than 0.001 mg C l^{-1}, until algae were added to restore the previous food concentration. The development of the animals and the morphological changes during growth were followed every 24 h using a microscope and determined according to Schrehardt (1987) from a sample of 77–142 individuals.

2.2. Algal cultures

I. galbana was grown in a semi-continuous culture (Reitan et al., 1994) at 24°C, using four 20 l vessels and a standard f/2 sea water medium (Guillard, 1975). Radioactive-labelled algae were prepared by adding 1 ml of isotope (20 μCi $NaH^{14}CO_3$, Dupont cat. no. NEC-086S), unlabelled $NaHCO_3$ corresponding to 1 g C l^{-1} and 5 ml of algal culture to 50 ml f/2 medium. The cultures were maintained in closed bottles and placed at a constant light intensity of 60 μEin m^{-2} s^{-1}. Before the algae were used in the feeding experiments they were centrifuged and washed twice in 10 ml filtered sea water at 3000 rpm for 5 min. The algal density was determined by measurements of absorbency (five replicates) at 750 nm by use of a spectrophotometer. Three samples (5 ml) from all suspensions were filtered through GFC filters for carbon analysis in an element analyser (Carlo Erba model 1106), to determine the carbon content, and thereby verify the measurements of absorbency. The deviations between these two methods were less than 8%.

The radioactivity of the food suspensions was determined from three samples of 0.1 ml added 100 μl 0.1 M hydrochloric acid and thereafter 3 ml scintillation cocktail (Opti-fluor, Packard). The specific activity of the algae (SA, ng C CPM^{-1}) was determined from the radioactivity measured in a scintillation counter (Packard liquid scintillation spectrometer model 199 TR) and the carbon content (Eq. (1))

$$SA = (\text{ng C ml}^{-1})/(\text{CPM ml}^{-1}) \quad (1)$$

2.3. Experimental design

Before each pulse meal with ^{14}C-labelled *I. galbana* the animals were carefully

sieved, washed in filtered sea water and transferred to beakers (250–400 ml, 0.5–11 ind ml^{-1}) with the same food concentration of ^{14}C-labelled *I. galbana*. The beakers were placed in a water bath at 26–28°C and the algal suspensions were occasionally agitated to keep the algae in homogenous suspension. The animals were incubated for 15 min, which is shorter than the gut passage time; the method is presented in detail by Evjemo et al. (1999).

After incubation in labelled algae (15 min) the animals were heat killed with hot water (>70°C), rinsed with filtered sea water and transferred to scintillation vials in numbers of 10 for digestion with 0.25 ml tissue solubiliser (Packard Soluene-350), prior to the addition of 3 ml scintillation cocktail (Packard Ultima Gold). In each sample the radioactivity of the animals was measured and converted to units of ingested carbon, (*I*, ingestion rate; ng C ind^{-1} h^{-1}), by use of Eq. (2)

$$I = \mathrm{SA} \cdot A_{\mathrm{cpm}} \tag{2}$$

where SA is the specific activity of the food suspension (SA, ng C CPM^{-1}, Eq. (1)) and A_{cpm} is the total net CPM in the animals after feeding on labelled algae (CPM ind^{-1} h^{-1}).

Preliminary experiments revealed that there was no significant difference in tracer content of heat-killed animals and of the animals plated alive in the scintillation vials ($P<0.001$). The background radioactivity was estimated from ten samples each with ten animals given unlabelled algae. These samples were compared with samples of animals fed for 2–5 s with labelled suspensions without detecting any significant differences ($P<0.001$). From all food suspensions, samples were taken to determine the specific activity and the particulate carbon content (three times 0.1 ml). All samples were counted for 30 min in the scintillation spectrometer. All ingestion rate data were corrected with a factor (f_c) because *A. franciscana* uses between 15 and 195 s (dependent on animal size) for handling the food particles before they are ingested (Evjemo et al., 1999). Consequently this will result in an underestimation of the ingestion rate and all ingestion-data in this study have been corrected according to the equation: $f_c = 15/(15 - \delta t)$, where (δt) is the handling time in minutes and 15 is the incubation time in minutes when the animals were feeding on labelled algae. This is described in detail by Evjemo et al. (1999) where f_c is determined from regression analysis.

One culture of *A. franciscana* was grown on squid meal. The density of nauplii was 2.2 ind ml^{-1} at day 1 decreasing to 1.6 ind ml^{-1} at the end of the experiment. During cultivation the nauplii were fed with 0.15 g squid meal l^{-1} day^{-1}. The food ration was gradually increased to 0.26 g l^{-1} at day 16 when the animals had reached post-larval stage. In order to reduce the bacterial growth when this diet was used the water was exchanged every day. Animals from this culture were used in the feeding experiments to see if any significant difference could be detected in the algal ingestion rate of *A. franciscana* grown on *I. galbana* or grown on squid meal. Different stages of animals from this culture were acclimated for 2 h at variable concentrations of *I. galbana* (0.2–20 mg C l^{-1}) before they were fed ^{14}C-labelled *I. galbana* at the same concentrations as during pre-feeding.

The dry weight and body length of *A. franciscana* was determined every 24 h. Dry weight (μg ind^{-1}) was calculated based on ten replicate samples from each culture. Live animals (5–20 individuals per sample, dependent on animal size) were transferred to pre-weighed tin capsules and kept at 60°C for 48 h. The dried capsules were weighed again, before calculating the dry weight, and thereafter analysed for carbon and nitrogen in an element analyser as described above. Body length (mm) was determined from live animals ($n=12$), rinsed with filtered seawater and placed ventrally on a Petri dish under a microscope. The length was measured as the distance from the posterior part of the animal to the anterior part of the post-mandibular and the post-maxillar region of young stages, or to the anterior part of abdomen in older stages. Animals with furca or with developing furca have this structure included in the total length (L, mm).

The survival rate (%) was determined daily in all cultures throughout the experimental period by calculating the mean density of animals from 12 subsamples (2.5–15 ml dependent on size of the animals).

2.4. Calculations

The relationships between body dry weight (W_{DW}) and carbon content (W_C) versus length are expressed through the power function (Eq. (3))

$$W = Ae^{(bL)} \tag{3}$$

where W is body dry weight or carbon content of *A. franciscana* (μg ind^{-1}), and L is the length (mm). The constants A and b were estimated by regression analysis.

The specific growth rate of *A. franciscana* (μ, day^{-1}) was calculated according to Eq. (4) (Rhee, 1978)

$$\mu = \{\ln(W_{n-1}/W_n)\}/t \tag{4}$$

where W_n and W_{n+1} are the mean dry weight of *A. franciscana* (μg C ind^{-1}) at day n and day $n+1$, respectively, and t the time interval in days.

The weight specific ingestion rate I_w (h^{-1}) was calculated from Eq. (5)

$$I_w = (I_n/C_n) \tag{5}$$

where I_n is the amount of food ingested h^{-1} at day n (ng C ind^{-1} h^{-1}) and C_n is the individual carbon content at day n (μg C ind^{-1}).

The production rate P_r (ng C ind^{-1} day^{-1}) of *A. franciscana* was calculated from Eq. (6)

$$P_r = dP/dt \tag{6}$$

where dP/dt is the increase of carbon (μg ind^{-1}) accumulated as body dry weight day^{-1}.

The conversion of food into biomass i.e., the relationship between the amount of biomass produced and the amount of food ingested during a define period of time (day^{-1}), was expressed as the yield, Eq. (7)

$$Y = W_n/I_n \tag{7}$$

where W_n and I_n are the increase of individual carbon content (μg C ind^{-1} day^{-1}), and the amount of ingested carbon (ng C ind^{-1} day^{-1}), respectively, in *A. franciscana* from day n to day $n+1$.

2.5. Statistics

To test differences between mean values of animals from different dietary groups all comparisons are based on Student's *t*-test (two-way). The general linear model procedure (GLM), SPSS Base 8.0 (analysis of covariance, ANCOVA), was used to compare and determine differences in the regression models. Data were log transformed where it was a necessary to satisfy homogeneity of variance, although non-transformed data are shown in the figures and tables.

3. Results

3.1. Growth of A. franciscana as a function of food concentration

The growth of *A. franciscana* fed at the highest food concentrations (10 and 20 mg C l^{-1}) increased exponentially whereafter it gradually levelled off after 8 days of cultivation, giving a sigmoidal-type growth curve (Fig. 1). Animals fed at the lower food concentrations (3, 5 and 7 mg C l^{-1}) did not reach this size, but became gradually bigger both as a function of time and food concentration, all through the growth period. *A. franciscana* fed at the lowest concentration (0.2 mg C l^{-1}) did not grow probably due to the low concentration of food particles. Animals from this culture were examined under a fluorescence microscope and few algal cells were observed in the gut (<51 cells per animal day^{-1}, $n=57$–82 animals per day). At day 5, more than 37% of the animals were dead, having lost 14–18% of their initial dry weight. Results from this culture are not included in the following figures and tables.

The effect of food concentration on individual growth, and on the mean dry weight of the animals, became more pronounced after 4 days of cultivation (Fig. 1). For example at day 3 the mean individual dry weight of the animals in all cultures ranged between 2.8 and 3.6 μg ind^{-1}. At day 7 and day 10, the mean dry weight ranged from 9.8 to 148 and 23.1 to 191 μg ind^{-1}, respectively. After 12 days of cultivation *A. franciscana* grown at the two highest food concentrations attained an individual dry weight of 211 and 195 μg ind^{-1} whereas the animals grown at the lower food concentrations, 3, 5 and 7 mg C l^{-1}, reached 29, 88 and 134 μg ind^{-1}, respectively, illustrating the substantial effect food concentration had on the growth of the animals (Fig. 1). There was no significant difference in mean individual dry weight of the animals fed the two highest food concentrations, 20 and 10 mg C l^{-1} ($P<0.001$). The body dry weight of the animals fed 3 and 7 mg C l^{-1} was, after 4 days growth, significantly lower compared with the two highest food concentrations ($P<0.005$). The results illustrate an increased growth of *A. franciscana* with increasing food concentration up to 10 mg C l^{-1}. Above this concentration the growth rate appeared to be constant. Consequently, food concentrations $\geq$10 mg C l^{-1} will in the following be considered as maximum food

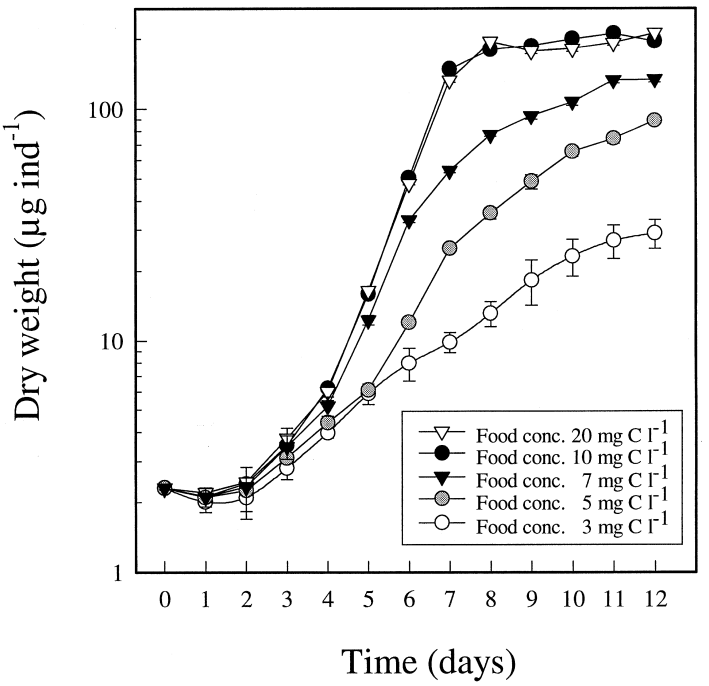

Fig. 1. Growth curves of *A. franciscana* fed *I. galbana* at five different concentrations from day 0 (newly hatched nauplii) until day 12. Data points represents mean dry weight (µg ind^{-1}) of ten replicates each with 5–20 animals (dependent on animal size).

concentrations, which may sustain maximum growth rate. The survival rate of *A. franciscana* when feeding on *I. galbana* was ≥92% at food concentrations ≥3 mg C l^{-1} throughout the experimental period (12 days).

3.2. Relationship between dry weight and length

The relationship between body dry weight and length of *A. franciscana* grown at five different food concentrations with *I. galbana* is shown in Fig. 2. Only data between day 1 and day 8 are included (see Fig. 1). All animals showed the same pattern of length versus dry-weight relationship ($r^2 = 0.98$, Table 1). The same was also found for the relationship between carbon content (µg C ind^{-1}) and length ($r^2 = 0.99$), giving parallel regression lines ($P < 0.001$) (Table 1, Fig. 2). The results suggest that the relationship between body dry weight, carbon content and length of 1–8 days old *A. franciscana* (0.84–5.0 mm) remained constant and was not affected by the food concentration, although the food concentration had a significant effect on the growth of the animals (Figs. 1 and 2).

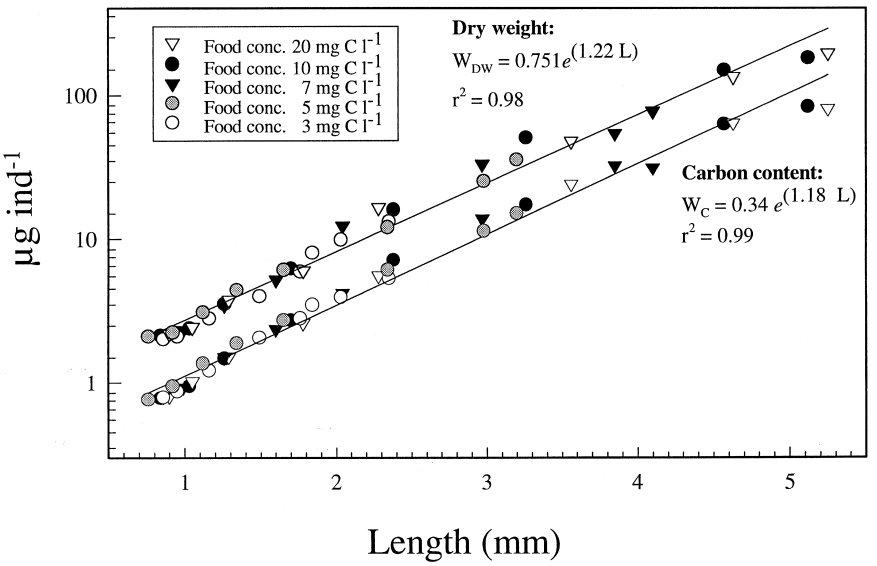

Fig. 2. Relationship between dry weight (W_{DW}, μg ind^{-1}) and carbon content (W_C, μg ind^{-1}) versus length (mm) of *A. franciscana* fed five different food concentrations of *I. galbana*. Regression data are shown in Table 1.

3.3. Carbon and nitrogen content

The body carbon and nitrogen contents (% of dry weight) of newly hatched nauplii were 45.5 and 9.7%, respectively. The nauplii grown at the maximum food concentrations shared an initial drop in the carbon content from hatching (day 0) until day 1, followed by an increase from day 1 to day 3. Thereafter both carbon and nitrogen contents remained relatively stable until the animals reached post-larval stage at day 11–12 (Table 2). The mean carbon and nitrogen contents of *A. franciscana* were 41.5±0.65% and 9.6±0.14% (mean±SE) of dry weight, respectively (Table 2). Animals grown at the lower food concentrations shared the same carbon and nitrogen content (% individual^{-1}) as the animals grown at the maximum food concentrations. Only at the lowest concentrations (3 and 5 mg C l^{-1}) a reduced carbon content was found between incubation (day 0) and day 5 (36.2–37.1%), before the carbon content increased reaching 40.8% at day 6 and remained >40% until day 12.

Table 1
Regressions coefficients of dry weight (W_{DW}, μg ind^{-1}) and carbon content (W_C, μg ind^{-1}) versus length of *A. franciscana* grown on different concentrations of *I. galbana*; animals were taken from each suspension every 24 h ($n=10$) each replicate with 5–20 animals

Equation	*A*	SE	*b*	SE	r^2
$W_{DW} = Ae^{bL}$	0.53	0.04	−0.11	1.24	0.98
$W_C = Ae^{bL}$	0.52	0.04	−0.51	1.33	0.99

Table 2
Carbon and nitrogen content in *A. franciscana* fed *I. galbana* (10 mg C l^{-1}). Samples were taken at regular time intervals during 12 days growth; Day 0 represents newly hatched nauplii

Day	Length (mm)	Dry weight (μg ind^{-1})	Carbon (μg ind^{-1})	Nitrogen (μg ind^{-1})	C/N ratio	% C ind^{-1}	% N ind^{-1}
0	0.63	2.3	0.93	0.20	4.7	45.5	9.7
1	0.84	2.1	0.77	0.19	4.1	36.2	8.9
2	1.1	2.4	0.95	0.22	4.3	39.4	9.1
3	1.3	3.5	1.5	0.37	4.1	43.3	10.5
4	1.7	6.2	2.7	0.6	4.7	42.9	9.2
5	2.4	15.9	7.0	1.4	4.9	43.9	9.0
6	3.3	50	20.7	4.9	4.2	41.2	9.8
7	4.6	148	59	14.4	4.1	40.0	9.7
8	5.0	180	74.8	18.5	4.0	41.5	10.3
9	5.2	186	79.3	17.4	4.6	42.6	9.3
10	5.8	200	80.6	20.2	4.0	40.4	10.1
11	5.8	210	85.8	20.3	4.2	40.8	9.7
12	5.9	195	80.5	19.5	4.1	41.2	9.9

3.4. Specific growth rate

The specific growth rate of *A. franciscana* (μ, day^{-1}, Eq. (4)) was dependent on the food concentration and the size of the animals (Fig. 3). The first 2 days after hatching

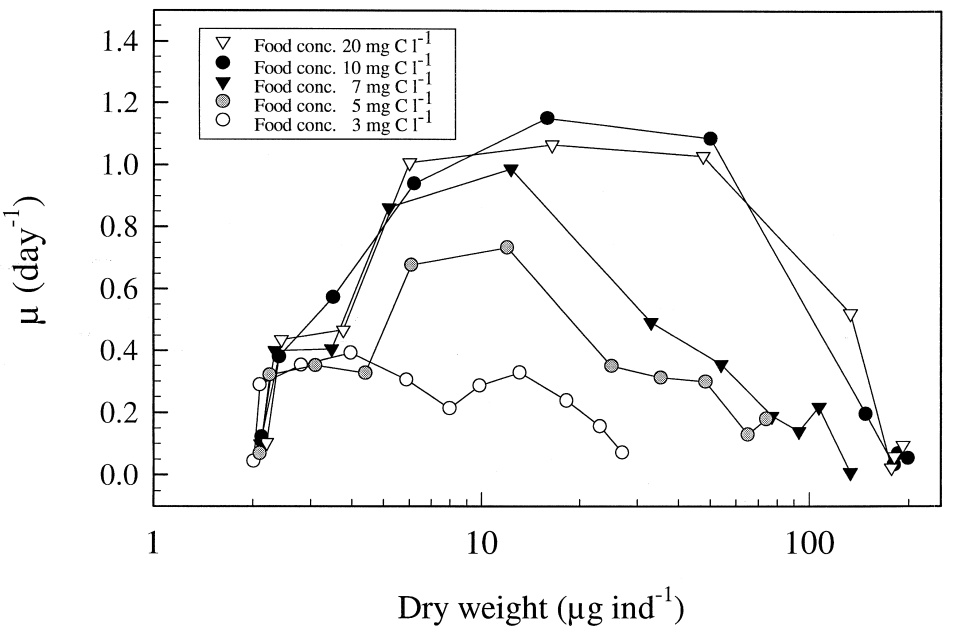

Fig. 3. Specific growth rate (μ, day^{-1}) of 2–12 days old *A. franciscana* grown at different food concentrations of *I. galbana* plotted as a function of animal dry weight.

the specific growth rate increased from 0.04 to 0.43 day^{-1} (animal weight 2–2.4 μg ind^{-1}, metanauplius IV), and the variation between animals maintained at different food concentrations was not significant. After day 3, the animals were >2.8 μg ind^{-1} (post-metanauplius I), and the specific growth rate started to increase with increasing food concentration and size of the animals (Fig. 3). Highest specific growth rate (1.19 day^{-1}) was obtained at day 7 for animals grown at the maximum food concentration (10 mg C l^{-1}), but the value was not significantly different from the animals fed at the highest food concentration (20 mg C l^{-1}, $P<0.001$). *A. franciscana* fed maximum food concentrations maintained a high specific growth rate until the animals reached 47–50 μg ind^{-1} at day 8 (post-metanauplius IV–VI). At the lower food concentrations (3, 5 and 7 mg C l^{-1}), the specific growth rate became successively lower as a function of food concentration (Fig. 3). Animals fed 5 and 7 mg C l^{-1} showed reduced growth rate from day 6 at a dry weight of 11–13 μg ind^{-1}. Animals fed 3 mg C l^{-1} exhibited specific growth rates in the range of 0.2–0.33 day^{-1}, and the values decreased to 0.07 day^{-1} at day 11. The results show that the minimum food concentration required for maximum growth was 10 mg C l^{-1}, and a further increase in the food availability did not result in a higher growth rate. Food concentrations below 10 mg C l^{-1} resulted in reduced growth rate. At a food concentration of 0.2 mg C l^{-1} the specific growth rate was negative (−0.18 day^{-1}), demonstrating that the food concentration was below the maintenance requirement of the animals.

3.5. Ingestion rate

The ingestion rate (Eq. (3)) of *A. franciscana* was correlated with the length of the animals at all food concentrations (Fig. 4), and increased linearly with increasing food concentration reaching a maximum level at 10 mg C l^{-1}. The ingestion rate of the animals feeding on 3, 5 and 7 mg C l^{-1} was significantly lower compared with the animals feeding on maximum food concentrations (ANCOVA, $P<0.001$). This implies that the ingestion rate was highly dependent on food concentration at levels <10 mg C l^{-1} were the ingestion rate decreased with decreasing food concentrations. At the higher concentrations (≥10 mg C l^{-1}) the ingestion rate was constant regardless of the concentration of food. *A. franciscana* cultivated on squid meal and thereafter short term acclimated (2 h) to different concentrations of *I. galbana* ingested the algae at the same rate as the animals fed with algae throughout the experimental period ($P<0.001$, Fig. 4), and it seems that *A. franciscana* have the ability to adapt rapidly to changes in the feeding regime. The survival rate in the culture fed squid meal was 78% after 16 days of cultivation.

In order to describe the relationship between ingestion rate, food concentration and animal size, all the ingestion-data, when *A. franciscana* was feeding on labelled *I. galbana*, were fitted to the equation

$$I = k(C) \cdot (L - k0)$$

where $k(C) = k1/[1 + k2 \cdot \exp(k3 \cdot C)]$. This equation has the desired properties: (1) for a given food concentration (mg C l^{-1}) I (ingestion rate, ng C ind^{-1} h^{-1}) will depend

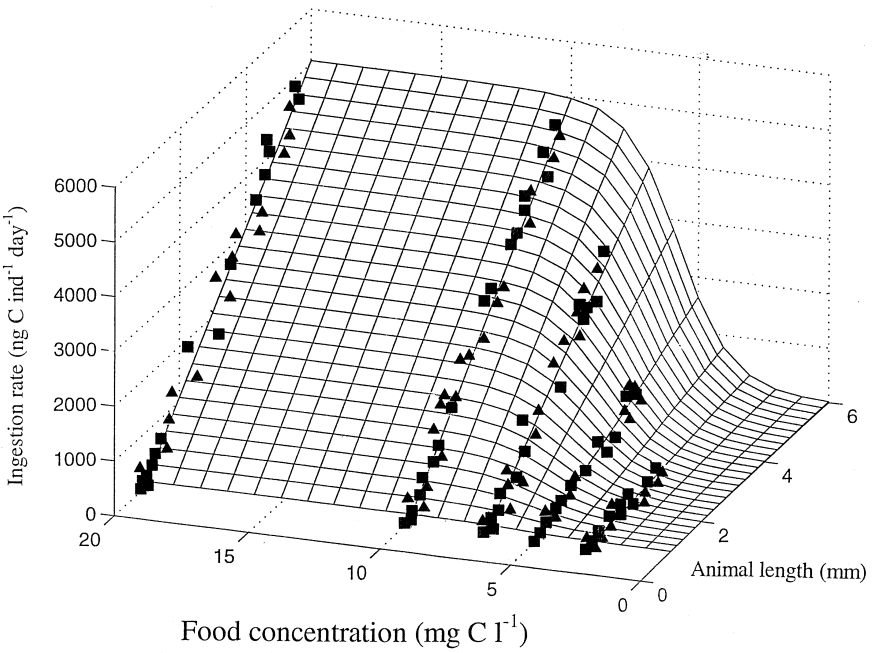

Fig. 4. Relationship between ingestion rate (I, ng C ind^{-1} h^{-1}), food concentration (mg C l^{-1}) and length (L, mm) of *A. franciscana* grown at five different food concentrations *I. galbana* (■), and *A. franciscana* grown on squid meal and acclimated to the desired concentrations of *I. galbana* for 2 h, before the animals were given ^{14}C labelled *I. galbana* (▲). All data point represents the mean of ten replicates, each with ten animals.

linearly on L (animal size, mm), (2) the proportionality factor $k(C)$ is a sigmoidal function of food concentration. Using a non-linear last squares procedure the constants $k0$, $k1$, $k2$ and $k3$ were calculated and the following equation generated $I = \{998/(1 + 270e^{-C})\}(L - 0.91)$.

3.6. Weight specific ingestion rate

The weight specific ingestion rate (I_w, h^{-1}, Eq. (5)) of the early stages of *A. franciscana* was not affected by food concentration, and the values increased almost at the same rate in all cultures from hatching until the animals had reached 2–2.3 μg ind^{-1} (Fig. 5). Thereafter, an optimum level was found for animals from all cultures before the weight specific ingestion rate successively decreased in the later developmental stages (Fig. 5). The optimum level obtained was highly dependent on the food concentration, and found for animals in the range of 3–20 μg ind^{-1}. The animals fed 10 and 20 mg C l^{-1} showed the highest values corresponding to a feed intake of 20–28% of the individual body carbon content h^{-1}. The weight specific ingestion rate was significantly lower for the animals feeding on 5 and 3 mg C l^{-1} ($P < 0.001$). At these concentrations the weight specific ingestion rate increased until the animals reached 4.5 μg ind^{-1}, whereafter it decreased steadily as a function of animal size.

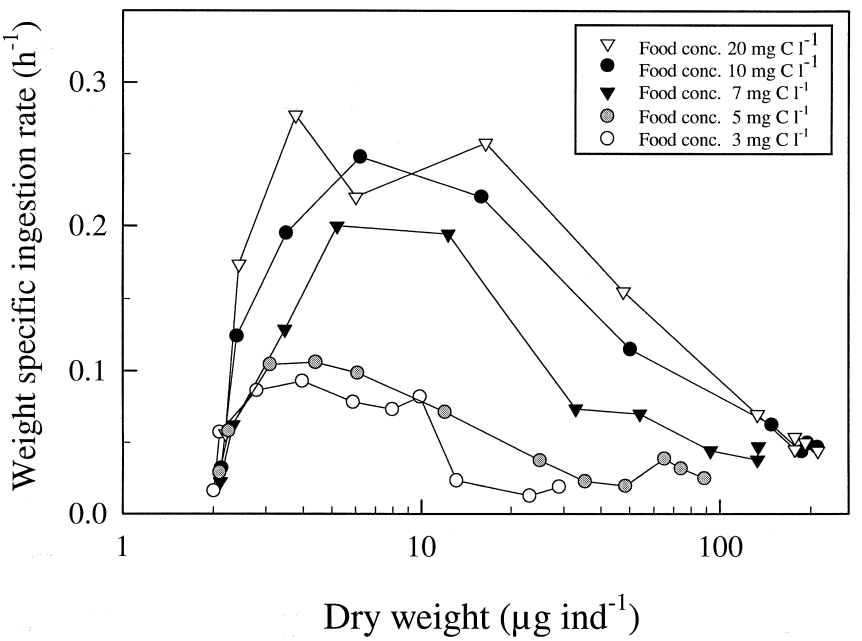

Fig. 5. Weight specific ingestion rate (I_w, h^{-1}) of 2–12 days old *A. franciscana* grown at five different concentrations of *I. galbana* plotted as a function of animal dry weight.

3.7. Production rate and yield

The production rate (P_r, ng C ind^{-1} day^{-1}, Eq. (6)) and the yield (day^{-1}, Eq. (7)) of *A. franciscana* was dependent on the developmental stage of the animals and the food concentration (Figs. 6 and 7). The smallest stages (metanauplius stages <5 µg ind^{-1}) was more or less unaffected by the food concentration whereas bigger animals (>6 µg ind^{-1}, post-metanauplius II) increased the production rate successively at various food concentration, and the values became significantly higher ($P<0.05$) for the animals grown at the two highest food concentrations (10 and 20 mg C l^{-1}). At these concentrations the animals had an optimum in the relative production rate (61–68%) until post-larval stages (I–II) was reached (Fig. 6B). The yield had an optimum when the animals reached post-metanauplius stages III–VIII (10–50 µg ind^{-1}) (Fig. 7). When the animal size exceeded 60 µg ind^{-1} (post-metanauplius VIII) the yield decreased reaching 0.05–0.1 when the animals reached preadult stage. At the lower food concentrations (7, 5 and 3 mg C l^{-1}) the production rate became successively lower (Fig. 6A and B) as a function of animal size. The animals grown at the lowest food concentrations (3 and 5 mg C l^{-1}) had the highest yield until post-metanauplius VI was reached (Fig. 7). This might indicate that these animals utilised a higher proportion of the ingested food for growth compared with animals grown at the maximum food concentrations.

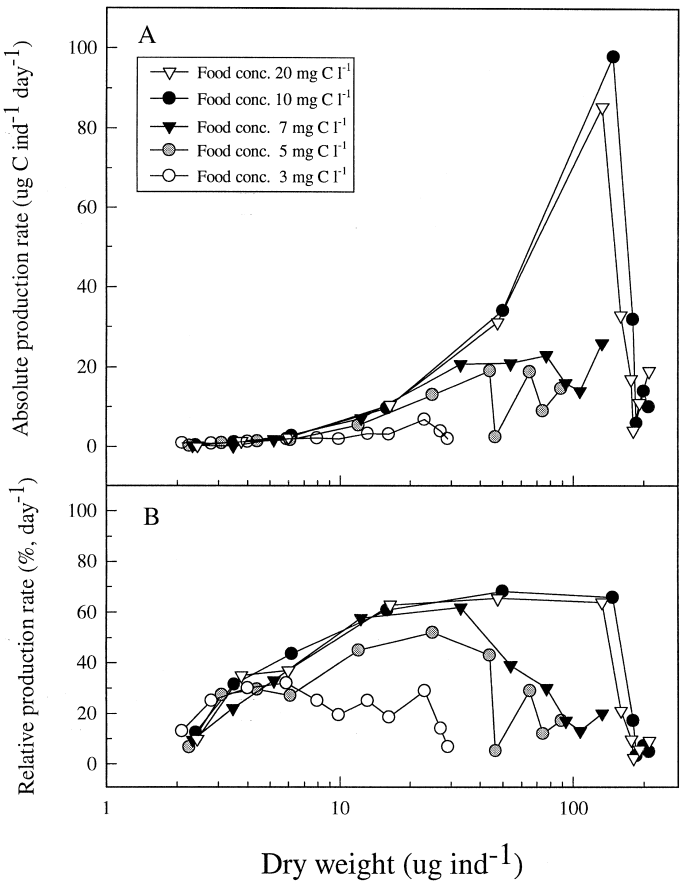

Fig. 6. (A) Relationship between absolute production rate (μg C ind^{-1} day^{-1}) and animal dry weight and (B) relative production rate (% day^{-1}) and animal dry weight of *A. franciscana* fed different concentrations *I. galbana*.

4. Discussion

4.1. Growth of A. franciscana at different food concentrations

When filter feeders such as *A. franciscana* are maintained under conditions of excess food they might attain maximum growth rate which is believed to be determined by temperature (Miller et al., 1977), animal density (<2 ind ml^{-1}, Abreu-Grobois et al., 1991), food concentration ($>41–233 \cdot 10^3$ cells ml^{-1}, Nimura, 1980), physical disturbance and water quality (Coutteau, 1992). For most species of the *Artemiidae* and different *Artemia*-strains the optimum temperature is in the range of 25–30°C (Sorgeloos et al., 1986). This means that *A. franciscana*, feeding on maximum food concentrations of *I. galbana* under laboratory conditions, most probably, grew at a rate

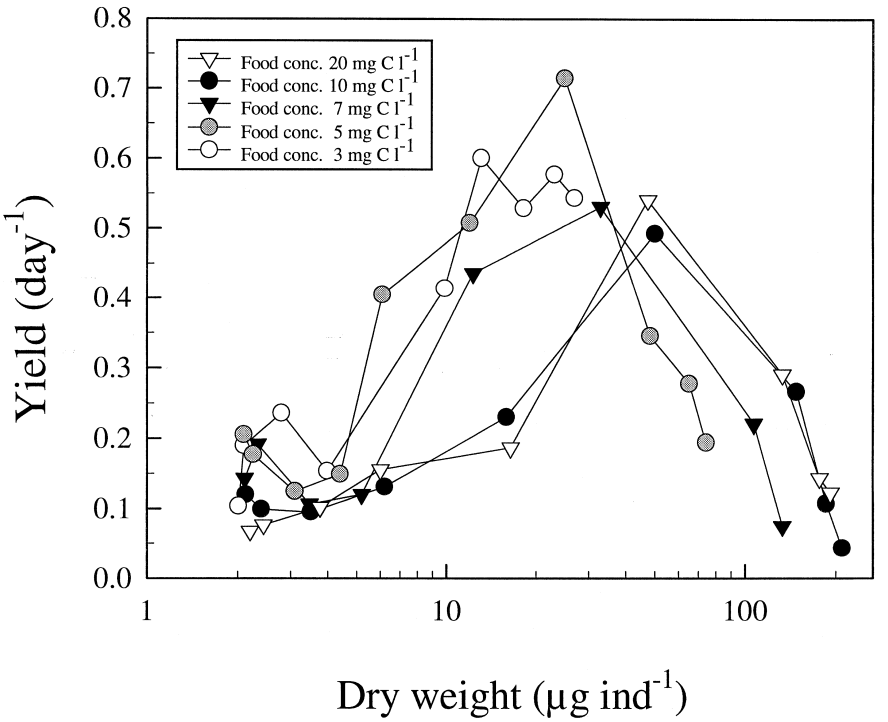

Fig. 7. Yield of 1–12 days old *A. franciscana* grown at five different concentrations of *I. galbana* plotted as a function of animal dry weight.

close to its maximum growth rate, reaching a dry weight of 180–194 µg ind^{-1} (postlarval stages (I–III), 5.9 mm) at day 8 (final density of animals 0.48–0.81 ind ml^{-1}). The digestibility and the nutritional value of the algae will, to some extent also affect the growth potential of the animals, but this has not been further evaluated in this study. Sick (1976) has shown that *A. franciscana* grown on five different species of marine algae, at a temperature of 25°C, showed significantly different survival, growth rate and assimilation rate. *Dunaliella viridis* gave highest growth rate of *A. franciscana*, but these values are 10–12% lower compared with the values obtained for *I. galbana*, and might be related to different diets and different temperatures. The animal density (ind ml^{-1}) could have reduced the growth rate during the first days of the growth period because initial density was 16–18 ind ml^{-1}. From incubation until day 4 the two highest food concentrations were reduced by 4–8%, and in order to keep the food concentrations as stable as possible the animal densities were diluted down to 1.1–2.4 ind ml^{-1} (day 4) reaching 0.48–0.81 ind ml^{-1} at day 12. In this period (day 4–12) animals were sampled from the cultures every 24 h due to dry weight, carbon/nitrogen analysis and for measurements of the ingestion rate in the feeding experiment with labelled algae. Animal density was also evaluated according to measurements on clearance rate of *A.*

franciscana [μl ind^{-1} h^{-1}; Evjemo et al. (1999)], and kept at a low level in order to avoid fluctuations in the food concentrations. Since the food concentrations were not reduced more than 2% between each feeding (every 12th hour) the animal density was not further reduced during the rest of the growth period. Other workers have reported similar densities of animals. At an original stocking density of 1–2 ind ml^{-1}, 9.4–9.8 mm *Artemia* were obtained after 10 days cultivation when feeding on *Dunaliella tertiolecta* (Abreu-Grobois et al., 1991), and in xenic cultures dominated by *Chlorella* sp. *Artemia* reached a mean size of 7.0 mm after 9 days at a density of 0.34 ind ml^{-1} (Rosowski, 1989).

The weight versus age curve for *A. franciscana* fed at the maximum food concentrations described adequately the growth of the animals from newly hatched nauplii, during different nauplius stages until post-larval stages (I–III) which, according to Schrehardt (1987), is characterised by the development of antenna and genital structures. At day 9 more than 72% of the animals had developed thoracopods X and XI with filter-seta which is a size-specific criterion for the post-larval stage (Schrehardt, 1987), and at day 10 the number of *A. franciscana* at this stage had increased to >83%. The same curve (Fig. 1) also indicates that the growth of *A. franciscana* was more influenced by the food concentration after day 4 when the animals had reached a length >1.34 mm and started the development of the thoracopods, which gradually contribute more in the feeding process (Schrehardt, 1987). These morphological changes make the animals gradually more efficient filter feeders with a better capacity to collect food particles and an increased ingestion rate (Fig. 4), leading to a higher growth rate. At all food concentrations an optimum in the specific growth rate was found when the size of the animals was in the range of 9–20 μg ind^{-1}, and the differences in specific growth rate between animals grown at different food concentrations became more pronounced after day 3, when the animals had reached a dry weight >4 μg ind^{-1} (Fig. 3). All these results indicate a close correlation between development stage and the growth rate of the animals, and shows that maximum growth of the animals was obtained only at food concentrations ≥10 mg C l^{-1} (maximum food concentrations). At the lower concentrations, the growth rate decreased as a function of the availability of food. Nevertheless, Coutteau (1992) found, when feeding *Artemia* on yeast, that maximal ingestion rate showed a sharp increase at the early life stages. As the animal size increased the maximum ingestion rate was reached at a gradually lower algal density.

The growth curve Fig. 1 also indicate that the growth of the animals fed at the highest food concentrations decreased when pre-adult stage was reached (length 5.2–5.8 mm; 186–210 μg ind^{-1}). It is well known that *A. franciscana* reach 800–900 μg ind^{-1} (length >10 mm) after less than 15 days (Rosowski, 1989; Abreu-Grobois et al., 1991). Recent findings (Evjemo, unpublished results) have shown that *A. franciscana* follows approximately the same growth curve as shown in Fig. 1, from day 1 until day 10, when grown under the same conditions (maximum food concentrations), and reach sexual maturity at a length of 10.4–11.6 mm (dry weight 890–1070 mg ind^{-1}) after 16 days. As shown in this study the specific growth rate (μ, day^{-1}) decreased from approximately 0.4–0.5 at a dry weight of 100 μg (day 7) to 0.1–0.2 at day 16. In that study the results also showed that the growth rate decreased when pre-adult stage was reached, but the growth curves of the animals in Fig. 1 fed the maximum food concentrations, are

lower than expected from day 10 until day 12. This might be related to the temperature, nutritional value of the algae or the physiological status of the animals.

The animals given 0.2 mg C l^{-1} did not grow, and the specific growth rate was found to be negative (-0.18 day^{-1}), indicating that the animals were starving. At the higher concentration (3 mg C l^{-1}) the animals had a growth rate >0.2 day^{-1} (animals in the range of 2.1–18.2 mg ind^{-1}), indicating that the maintenance requirement of food for *A. franciscana*, i.e., the food concentration were the animals neither gain nor loose weight, is in the range of 0.2–3 mg C l^{-1} when grown on *I. galbana*. This might be different when the size of the animals increase. As shown in Fig. 1 the animals grown at food concentration 3 mg C l^{-1} gradually increased the dry weight but the specific growth rate decreased and did not reach an optimum as in the other cultures (Fig. 3). On the other hand the yield (Fig. 7) was higher for the animals in this culture and might indicate an increased utilisation of the food at low concentrations when animal size increases. For other planktonic crustacean like copepods and *Daphnia* sp., 0.2 mg C l^{-1} is a concentration of food, which may sustain both maximum ingestion and growth rate. For instance copepods can grow and reproduce at concentrations <0.05 mg C l^{-1} (Harris and Paffenhöfer, 1976; Vidal, 1980; Williamson et al., 1985; Piyasiri, 1985) and *Daphniids* appear to have consistently higher values of the incipient limiting concentration than copepods, 0.2–0.4 mg C l^{-1} (Geller, 1975; Porter et al., 1982; Taylor, 1985; Bohrer and Lampert, 1988).

4.2. Carbon and nitrogen content

The carbon content of *A. franciscana* (% of dry weight) was found to be quite stable (Fig. 2), and independent of the growth conditions (36.2–45% of dry weight). Oppenheimer and Moreira (1980) have, on the other hand, found striking differences in the carbon content for different developmental stages of *Artemia* (27.5 to 55.6% of dry weight), using a strain from San Francisco, CA, USA. In both studies, however, the variation was most pronounced when the nauplii molts from instar I into instar II and Oppenheimer and Moreira (1980) relates these changes to the period of 'self-absorption', when the nauplii change from endogenous metabolism of the yolk reserve (nauplius), to actively filtering food particles from the food suspension (metanauplius I). The variation in nitrogen content ranged between 8.4 and 10.5% in both studies, but an initial drop in the nitrogen content, as was found for the carbon content, was only seen in this study (Table 2).

4.3. Ingestion rate A. franciscana

The ingestion rate increased with increasing food concentration and size of the animals. At the highest food concentrations (10 and 20 mg C l^{-1}) no significant difference was obtained ($P<0.001$) (Fig. 4), and only the size of the animals affected the ingestion rate. Compared with the ingestion rate at the lower concentrations (≤ 7 mg C l^{-1}) all developmental stages of *A. franciscana* had a significantly lower ingestion rate ($P<0.001$) (Fig. 4). At these concentrations both the animal size and the food

concentration affected the ingestion rate (Fig. 4). These results are in close agreement with the Type 3 functional response curves, presented by Evjemo et al. (1999), where the ingestion rate is plotted as a function of food concentration and follows a sigmoidal curve showing maximum and constant ingestion rate at food concentrations $\geq$10 mg C l^{-1}. Those findings are well reflected in the data presented in Fig. 4 and in the equation $I = \{998/(1 + 270e^{-C})\}(L - 0.91)$, describing the relationship between ingestion rate (I), food concentration (C) and animal size (L, mm). The equation gave very close correlation between the predicted and the measured values (Fig. 4).

For several species of filter feeding zooplankton a critical food concentration has been detected, often referred to as the incipient limiting concentration (ILC) (Rigler, 1961). Above this concentration the animals ingest food at a maximum rate and the food concentrations are said to be saturated (Mullin et al., 1975), corresponding to the maximum food concentrations in this study. Below the ILC the ingestion rate decreases with decreasing food concentration (McMahon and Rigler, 1963; McMahon, 1965). The incipient limiting concentration for different stages of *A. franciscana*, when feeding on *I. galbana*, is in the range of 5–7 for metanauplius stages ($L = 1.1$ mm) increasing to 9–9.5 mg C l^{-1} for the post-larval stages ($L = 5.2$ mm) (Evjemo et al., 1999). Those findings support the results obtained in this study were both the growth rate and ingestion rate (Figs. 1, 3 and 4) were lower for animals feeding on food concentrations $\leq$7 mg C l^{-1}, compared to the animals feeding on the maximum food concentrations ($\geq$10 mg C l^{-1}). At day 9 the developmental stages of the animals grown at these two food concentrations (7 and 10 mg C l^{-1}) were significantly different ($P < 0.001$). From a sample of 266 individuals less than 13% had reached post larval stage (culture grown at 7 mg C l^{-1}) whereas more than 88% of the animals grown at 10 mg C l^{-1} had reached post larval stage ($n = 293$). All these findings support that 7 mg C l^{-1} is below the maximum food concentrations when *A. franciscana* is feeding on *I. galbana*.

The weight specific ingestion rate (I_w, Fig. 6) also revealed that the animals grown at the maximum food concentrations ingested the highest amounts of food, and showed maximum values corresponding to a feed intake equal to 20–28% of individual carbon content per hour. This means that considerably amounts of food were passing through the animal's gut virtually undigested. Observations from fluorescence microscope showed high amounts of undigested algal cells in the faecal pellets from animals feeding at concentrations $\geq$10 mg C l^{-1}. The high ingestion rate could be related to the digestibility of the algae. Coutteau (1992) has shown that the digestibility of food particles affects both the ingestion rate and the assimilation efficiency. When feeding *A. franciscana* with different concentrations of a diet having high digestibility the ingestion rate was lower and the assimilation rate was higher, and nearly constant, compared to the animals giving a diet with low digestibility. In the latter assimilation efficiency decreased considerably as a function of food concentration. Similar findings have been demonstrated by Evjemo et al. (1999) showing that the assimilation efficiency for *A. franciscana,* feeding on *I. galbana,* decrease when food concentrations increase (a reduction of the assimilation efficiency from 88 to 38%, at food concentrations ranging from 0.5 to 30 mg C l^{-1}). To compensate for the low density of food particles a higher fraction of the ingested food is probably conversed into biomass at low concentrations (3–7 mg C l^{-1}), and this might explain why the yield (Fig. 7) was higher for the

animals feeding on low concentrations compared to the maximum food concentrations (when animal size <40 μg ind^{-1}). At the maximum food concentrations the ingestion rate was considerably higher (Fig. 4) and only a minor fraction of the ingested food must be assimilated from the gut in order to maintain a high growth rate.

The production rate was affected by the food concentration only at concentration <10 mg C l^{-1} and when animal size was >7 μg ind^{-1}. We have not considered losses due to moulting, respiratory losses or other forms of excreted carbon that affect the production rate and the data in Fig. 6 might be slightly underestimated. Production rates reported for the *Cladoceran* varies with environmental conditions, but production rates $>70\%$ are reported by Kryutchkowa and Sladecek (1969) and Richman (1958). In this study the relative production rate (%, day^{-1}) reached an optimum (61–68%) when the animals size was in the range of 20–148 μg ind^{-1}. Animals grown at the lowest food concentration had the lowest production rate (animals in the range of 7–30 μg ind^{-1}), and to compensate for the low concentration of food particles, the animals utilised probably a higher amount of the ingested food for growth, giving a higher yield (Fig. 7).

The results from this study indicate that food concentration is a very important ecological parameter for the growth and production rate of *A. franciscana*. According to literature this is well established for both cladoceran and copepods, like in the papers from Lampert, 1977a, Porter et al., 1982, Mullin, 1963, Mullin and Brooks, 1976, Miller et al., 1977 and Vidal, 1980. Regarding the food concentration for filter feeding zooplankton, one must consider that in the ocean, in fjords and in many lakes and ponds the food concentration (mg C l^{-1}) are normally in the range of 0.1 to 1.0 mg C l^{-1} (Wetzel, 1983; Sakshaug and Olsen, 1986). In the natural habitat of *A. franciscana* the food concentration is considerably higher, reaching $250 \cdot 10^6$ cells l^{-1} of *Dunaliella* sp. (Stephens and Gillespie, 1976), and *A. franciscana* are probably adapted to relatively high food concentrations. For *I. galbana* the number of cells, $250 \cdot 10^6$ cells l^{-1}, corresponds to 2.6 mg C l^{-1} (Reitan et al., 1993), but the number of cells per volume of these two algal species cannot be directly compared since *Dunaliella* sp. is a considerably larger cell (Throndsen, 1997), with a greater cell volume. This will lead to a higher food concentration (mg C l^{-1}) of *Dunaliella* sp. than the same cell number per volume of *I. galbana*, and might indicate that *A. franciscana* is adapted to higher food concentration compared to other planktonic crustacean.

Adaptations to different food concentrations is probably the reason why there is a relatively large difference in the food concentrations where cladoceran and copepods can grow, compared to the results from our study on *A. franciscana*. It is also interesting to notice both from our findings and other studies (Brune and Anderson, 1989; Rosowski, 1989; Abreu-Grobois et al., 1991; Nimura et al., 1994) that it seems as if the *Artemiidae* also are adapted to a short growth cycle, with high growth rate in a short period of time, if the availability of food is at a relatively high level.

5. Conclusions

The growth rate of *A. franciscana* was influenced by the food concentration and the size of the animals. The specific growth rate (μ, day^{-1}) was highly affected by the food

concentrations, however, an optimum was found at all concentrations when the animal size was in the range of 6–50 μg ind^{-1}. Minimum food concentration for maximum growth and production rate was 10 mg C l^{-1}. Higher concentrations of food had no effect, but lower concentrations reduced both the growth and production rate.

A. franciscana given 0.2 mg C l^{-1} did not grow. At 3 mg C l^{-1} the animals had a growth rate >0.2 day^{-1} indicating that the maintenance requirement of food is in the range of 0.2–3 mg C l^{-1} when grown on *I. galbana*.

Both the ingestion rate (ng C ind^{-1} h^{-1}) and the weight specific ingestion rate (h^{-1}) increased as a function of food concentration and reached an optimum at concentrations $\geq$10 mg C l^{-1} (maximum food concentrations). Further increase of the algal concentration did not affect the ingestion rate.

The yield had an optimum when the animals were in the range of 10–50 μg ind^{-1}. Highest yield was found for the animals grown at relatively low food concentrations (below the maximum food concentrations). The results might indicate that these animals utilised a higher proportion of the ingested food for growth compared with the animals grown at the higher concentrations.

Acknowledgements

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